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(54) Title: PHARMACEUTICAL COMPOSITION COMPRISING CLIOQUINOL AND VITAMIN B <sub>12</sub>			
(57) Abstract <p>A new pharmaceutical composition is disclosed that comprises clioquinol, vitamin B<sub>12</sub>, and, optionally, pharmaceutical acceptable carriers and/or excipients. The use of the pharmaceutical composition removes or alleviates the side effects of clioquinol.</p>			

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PHARMACEUTICAL COMPOSITION COMPRISING CLIOQUINOL AND  
VITAMIN B<sub>12</sub>.

INTRODUCTION

5       The present invention relates to a pharmaceutical composition comprising clioquinol and vitamin B<sub>12</sub> as well as the use of vitamin B<sub>12</sub> and clioquinol for the manufacture of a pharmaceutical composition for the treatment of a disorder that responds to clioquinol  
10 administration. The present invention is also directed to methods of treatment and prevention of disorders responsive to clioquinol administration, including but not limited to Alzheimer's disease, Parkinson's disease, *H. pylori* infections/ulcers, and other infectious  
15 diseases by administering both the chelating agent clioquinol and vitamin B<sub>12</sub>. The use of vitamin B<sub>12</sub> in combination with clioquinol inhibits detrimental side effects of clioquinol administration.

20

BACKGROUND OF THE INVENTION

Clioquinol and SMON

Clioquinol has been formulated and administered in various ways, at various doses (Ozawa, et al., 1986, Acta Neuropathol (Berl) 69: 272-277; Yamanaka, et al.,  
25 1973, J. Biochem., 73, 993-998; Sobue, et al., 1971, Neurology, 21: 168-173; Tamura, et al., 1973, Clinica Chimica Acta., 47: 13-20; Jack and Riess, 1973, J. Pharm. Sci., 62(12): 1929-1932; Baumgartner, et al., 1979, Journal of Neurology, Neurosurgery, and Psychia-  
30 try 42: 1073-1083, Kono, 1975, Japan, J. Med. Sci. Biol. 28: 1-21; Sargeant and Lumsden, 1976, Transactions of the Royal Society of Tropical Medicine and Hygiene; 70(1): 54-56; Nakae, et al., 1973, Lancet, 171-173; Mumenthaler, et al., 1979, Journal of Neurol-  
35 ogy, Neurosurgery, and Psychiatry 42: 1084-1090;

Schmid, et al., 1973, *Arzneim.-Forsch. (Drug Res.)* 23(11): 1560-1566; Oakley, 1973, *JAMA* 225(4): 395-397; David, et al., 1943, *American J. Trop. Med.*, 24: 29-33; Thomas, et al., 1984, *Journal of the Neurological Sciences*, 64: 277-295; Yoshimura, 1992, *Molecular and Chemical Neuropathology*, 16: 59-84; Nakae, 1974, *Japan Public Health Journal*, 15: 607-611; Tateishi, 1973, *Japan Public Health Journal*, 15: 187-196; Degen, et al., 1979, *Dermatologica* 159: 295-301; Goto, et al., 10 1982, *J. Toxicological Sciences*, 7: 1-12).

Clioquinol (5-chloro-7-iodo-8-hydroxyquinoline) was previously frequently used for the treatment of various disorders, such as amoebiasis and non-specific infectious diarrhea (Kono, 1975, *Japan J. Med. Sci. Biol.*, 28: 1-19, Meade, 1975, *Brit. J. prev. soc. Med.*, 29: 157-169). However, the use of clioquinol was stopped in Japan when the Japanese Government officially banned the sale in September 1970. The ban was motivated by the presumption that clioquinol caused 20 subacute myelo-optico-neuropathy (SMON). Subsequently, clioquinol was withdrawn from the market in most other countries of the world on the recommendation of the World Health Organisation. At present, clioquinol is only used topically due to its antibacterial and anti-25 fungal activity in skin infections.

SMON develops with an acute or subacute onset preceded by abdominal disorders and is characterised by dysesthesia of the legs, sensory disturbances, a variable degree of motor weakness, and visual loss. 30 Corresponding to these clinical findings, SMON reveals pathologically symmetrical degeneration in peripheral nerves, spinal cord, posterior column, cortico-spinal tract, and optic nerves.

The occurrence of SMON was confined to Japan even 35 though clioquinol was prescribed world-wide and not

only in Japan. In the published literature no other systematic pathological features resulting from the administration of clioquinol have been described other than the cases of SMON in Japan.

5        Although, in March 1972, the SMON Research Commission in Japan established a guideline for the treatment of SMON, wherein vitamin B<sub>12</sub> was recommended to be administered as part of a supply of various vitamins (Jap. Med. Sci. Biol. 28 Suppl. (1975)), it was never  
10 recognized that the deficiency of vitamin B<sub>12</sub>, at least to some extent, might be responsible for SMON. Actually, the effect of vitamin B<sub>12</sub> in the treatment of SMON has been contested by Okuda, K. ("On vitamin B<sub>12</sub> metabolism in SMON patients years after the onset."  
15 Report of SMON Research Commission in 1972 (1973) 86), who reported that the vitamin B<sub>12</sub> level in the serum of SMON patients is normal. It was also observed that there may be pathological differences between SMON and B<sub>12</sub> deficiency. (Ricoy, et al., 1982, J. Neurol. Sci.,  
20 53: 241-251.) Hence, it was supposed that the administration of vitamin B<sub>12</sub> would not produce any improvement in the symptoms and signs of SMON.

After the withdrawal of the clioquinol from the market there was a dramatic disappearance of new cases  
25 of SMON.

Renewed interest has been evinced in clioquinol recently as it has been shown to be effective in the treatment of *Helicobacter pylori* (see International Publication No. WO 95/31199, dated November 23, 1995 of  
30 the present applicant) and neurotoxic injury (see International Publication No. WO 97/09976, dated March 29, 1997 of Washington University). In another filed, but not yet published, patent application, of the present applicant, clioquinol is disclosed to have  
35 activity in the treatment of Alzheimer's disease

(PCT/IB97/00983, filed August 8, 1997). Further, it is supposed that clioquinol has activity in the treatment of Parkinson's disease. The aforementioned applications and publications are incorporated herein by reference 5 in their entireties.

#### Alzheimer's Disease

Alzheimer's disease (AD), which is the single major cause of dementia in adults in industrialized 10 societies, is a degenerative brain disorder characterized clinically by a progressive loss of memory, confusion, dementia and ultimately death. Histopathologically, Alzheimer's disease is characterized by the presence in the neocortex, especially the hippocampus, 15 of two brain lesions: the neurofibrillary tangles (NFTs) of paired helical filaments (PHF) in the neurons and the neuritic (senile) plaques in the extracellular space. The formation of senile plaques is related to the appearance of the symptoms and signs of the disease, including amnesia. After the formation of senile 20 plaque, neurofibrillary tangles are produced in the neuronal bodies. The formation of neurofibrillary tangles is related to the worsening of amnesia and of the other symptoms of dementia.

25 A major component of the plaques is amyloid deposits, which are polypeptides referred to herein as A $\beta$  (Amyloid-beta). The normal function of A $\beta$  is not known at present but might be to form cation selective channels across cell membranes (Kawahara M. et al., 30 1997, Biophysical Journal 73/1, 67-75).

The precipitation of synthetic A $\beta$  has been shown to be caused by several environmental factors including low pH, high salt concentrations and the presence of metals, e.g. zinc, copper, and mercury (Bush, A.I. et 35 al., 1995, Science 268: 1921-1923). It has been report-

ed that A $\beta$  itself specifically and saturably binds zinc with a high affinity binding ( $K_D = 107$  nM) at a molar ratio of 1:1 (zinc: A $\beta$ ) (Bush, A.I. et al., 1994, J. Biol. Chem. 269: 12152-12158). This binding takes place  
5 at physiological concentrations of zinc (Bush, A.I. et al., 1994, Science 265: 1464-1467).

The chelating ability of clioquinol is known for Fe, Co, Ni and Zn (Kidani Y et al., 1974, Jap. Analyst 23: 1375-1378). Using mass spectrophotometry the co-  
10 ordination number for clioquinol in case of Co(II), Ni(II), Cu(II) and Zn(II) is 2, whereas the coordination number for Fe(III) is 3. Reportedly, injected preparations of clioquinol have crossed the blood-brain-barrier, leaving concentrations thereof in the  
15 brain on the order of 20  $\mu$ l/ml when administered at dosages of 10-20 mg/kg (Tateishi J. et al., 1973, Psychiat. Neurol. Jap. 75: 187-196 and Tamura Z, 1975, Jap. J. Med. Sci. Biol. Suppl. 28: 69-77). The concentration of clioquinol was also found to be high in such  
20 areas of the brain as the hippocampus.

#### Parkinson's Disease

In patients with typical Parkinson's disease (PD), the results of magnetic resonance imaging are usually  
25 normal. However, when a high field strength, heavily T2 weighed magnetic resonance imaging is used, a wider area of lucency in the substantia nigra is seen, indicative of increased accumulation of iron. In general, iron in the substantia nigra area has been  
30 reported in many publications as being causative of or at least contributing in the development of Parkinson's disease.

Iron is known to be a catalyst for the fenton reaction, by which the generation of reactive oxygen  
35 free radicals occurs. Iron is typically found within

cells in the iron storage molecule ferritin. Once stored in ferritin, iron is unavailable as a catalyst for the production of free radicals.

Despite the enormous therapeutic advances, Parkinson's disease continues to be one of the most common causes of disability in the elderly. Research has been focused on the MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine toxin) model of Parkinson's disease based on the advances in the neuropathology and neurochemistry of the disease.

This toxin selectively damages the dopaminergic neurones of the substantia nigra when oxidized to MPP+. Anti-oxidants prevent the oxidation of MPTP to MPP+ and have been investigated clinically for the treatment of Parkinson's disease. It has been also postulated that endogenous or exogenous toxins, including metabolites of dopamine, generate hydroxyl radicals and hydrogen peroxide, which are known neurotoxic substances. These toxins interfere with normal mitochondrial metabolism. Abnormalities have also been reported in the complex I (NADH: ubiquinone oxidoreductase).

Citation or discussion of a reference hereinabove shall not be construed as an admission that such is prior art to the present invention.

25

#### SUMMARY OF THE INVENTION

According to the present invention a new pharmaceutical composition is provided, which comprises clioquinol, vitamin B<sub>12</sub>, and, optionally, pharmaceutically acceptable carriers.

Also the use of vitamin B<sub>12</sub> and clioquinol for the manufacture of a pharmaceutical composition for the treatment or prevention of a disorder responding to clioquinol administration is suggested. Such disorders include, but are not limited to, Alzheimer's disease,



Parkinson's disease, and bacterial or fungal infection, particularly bacterial infection caused by *Helicobacter pylori*.

Another object of the present invention is to provide methods of treating or preventing a disorder therapeutically responsive to clioquinol administration by administering a combination of clioquinol and vitamin B<sub>12</sub>.

Such disorders include, but are not limited to Alzheimer's disease, Parkinson's disease, and fungal protozoal, viral or bacterial infection, including but not limited to bacterial infection caused by *Helicobacter pylori*.

The invention also provides methods of treating or preventing undesirable side effects of clioquinol administration by also administering to the subject receiving clioquinol, concurrently or sequentially, vitamin B<sub>12</sub>.

## BRIEF DESCRIPTION OF THE FIGURES

Figure 1 depicts the NMR spectra of three solutions. Solution a contained vitamin B<sub>12</sub> (hydroxycobalamin), at a concentration of 2.6 mM. Solution b contained a mixture of 2.6 mM vitamin B<sub>12</sub> and 10 mM clioquinol glucuronide (mole ratio of about 1:4). Solution c contained 10 mM clioquinol glucuronide.

Figure 2 depicts the right half of Figure 1 expanded for easier comparison of the resonance positions.

Figure 3 depicts the left half of Figure 1 expanded for easier comparison of the resonance positions.

Figure 4 depicts the amounts of  $\beta$  amyloid found in the supernatants of brain specimen homogenates taken from patients diagnosed with Alzheimer's disease after

the homogenates were exposed to several concentrations of clioquinol (100% = 800nM clioquinol). The lane labelled "1-40" contains TCA precipitated synthetic A $\beta$ .

5                   **DETAILED DESCRIPTION OF THE INVENTION**

In view of the efficacy of clioquinol in therapy and prophylaxis of various diseases and disorders, there is a need to reintroduce clioquinol into the market. In this respect it is important to avoid the  
10 side effects of clioquinol administration, such as the symptoms and signs of SMON.

The relationship between SMON and clioquinol has been investigated and an explanation of the development of SMON as a consequence of clioquinol administration  
15 has been discovered. Thus, the object of the present invention is to remove or alleviate or prevent at least some of the side effects that occur when clioquinol is administered, whatever the reason for the administration may be. This object is achieved through the co-  
20 administration of vitamin B<sub>12</sub> with clioquinol.

In the following the invention will be explained in further detail. The proposed mechanism of action of the invention is not intended to limit the invention to said mechanism.

25       It is known that clioquinol is excreted through the kidneys as glucuronide or sulphate derivatives (Kotaki H., et al.: "Enterohepatic circulation of clioquinol in the rat", J. Pharmacobiodyn. 1984 June; 7(6): 420-5 and Jurima M. et al.: "Metabolism of 14C-  
30 iodochlorhydroxyquinoline in the dog and the rat", J. Pharmacobiodyn. 1984 March; 7(3): 164-70), e.g. as the compound methyl(5-chloro-7-iodo-quinolyl-2',3',4'-tri-O-acetyl-glucopyranosid)uronate. For short, this compound is referred to as clioquinol glucuronide in  
35 the following.

The detoxification of hydrophobic substances, such as clioquinol, in the body predominantly occurs in the liver. Therefore, it is believed that the clearance of clioquinol happens as follows: Clioquinol is converted to clioquinol glucuronide in the liver. Following the formation, the water soluble clioquinol glucuronide is secreted to the bile. The bile enters the intestine, wherein a major amount of the clioquinol glucuronide is evacuated in the stool. A certain amount of the clioquinol glucuronide is resorbed from the intestine to the blood. The clioquinol glucuronide is filtered from the blood in the kidneys and appears in the terminal urine.

By treating mice with clioquinol and subsequently administering a radioisotope of vitamin B<sub>12</sub> ([<sup>57</sup>Co]-cyanocobalamine), it is shown in Example 1 below that the concentration of vitamin B<sub>12</sub> in the brain and the liver of the clioquinol-treated mice remains at a normal level, whereas the concentration of the radioisotope of vitamin B<sub>12</sub> is decreased in the kidney of such mice compared to the normal level. This finding suggests a metabolism of vitamin B<sub>12</sub> being dependent on clioquinol. Further, the finding suggests that the kidneys are the target organs, wherein the clioquinol dependent metabolism occurs.

In order to investigate a possible interaction between clioquinol glucuronide and vitamin B<sub>12</sub>, an experiment was designed, wherein clioquinol glucuronide and vitamin B<sub>12</sub> were mixed in water. The mixture was analyzed by <sup>1</sup>H NMR. The <sup>1</sup>H NMR spectra, see Figs. 1-3, show that some of the resonances of vitamin B<sub>12</sub> (corresponding to the benzimidazole moiety) have shifted, and the same is observed for two resonances of the clioquinol glucuronide (corresponding to the quinoline moiety). It is believed by applicant that similar

results would be expected using free clioquinol, however clioquinol cannot be dissolved in aqueous solutions for NMR testing.

The results indicate a hydrophobic interaction  
5 between clioquinol glucuronide and vitamin B<sub>12</sub>, possibly between the benzimidazole moiety of the vitamin B<sub>12</sub> and the quinoline moiety of clioquinol glucuronide.

Vitamin B<sub>12</sub> is normally resorbed actively from the renal plasma after it has been filtered. In that way  
10 the body recovers most of the vitamin B<sub>12</sub> that would otherwise have been lost in the urine. It has recently been demonstrated that the resorption of vitamin B<sub>12</sub> is mediated by the action of the membrane protein megalin (Moestrup S.K. et al. Proc. Natl. Acad. Sci. 1996;  
15 93(16): 8612-7). The megalin is shown to have a strong affinity towards the binding of a complex formed by vitamin B<sub>12</sub> and the transport protein transcobalamin.

Based on the new finding reported herein, viz. that vitamin B<sub>12</sub> binds to clioquinol glucuronide, it is  
20 believed that vitamin B<sub>12</sub> does not bind to the megalin protein and/or the transcobalamin when it is already bound to the clioquinol glucuronide. Thus, the resorption of vitamin B<sub>12</sub> will fail and the body will suffer from vitamin B<sub>12</sub> deficiency after a certain time  
25 period of clioquinol administration if the body is not supplied with enough new vitamin B<sub>12</sub> through the normal diet.

Since the only source of vitamin B<sub>12</sub> is microorganisms only microorganisms and species eating microor-  
30 ganisms contains vitamin B<sub>12</sub>. Thus, diets low in meat and/or microorganisms will evidently cause vitamin B<sub>12</sub> deficiency. If persons are supplied with diets with a too low content of vitamin B<sub>12</sub> the administration of clioquinol will worsen the condition as the resorption  
35 of B<sub>12</sub> is prevented by the competitive binding of

clioquinol with megalin. The fact that the Japanese diet in the 1960s predominantly consisted of vegetables and cereals, especially rice, (Kromhout D, et al : "Food consumption pattern in the 1960s in seven countries", 5 Am.J. Clin. Nutr. 49: 889-894, 1989) may explain why the SMON disease was confined to the Japan.

The present invention solves the problem of clioquinol induced vitamin B<sub>12</sub> deficiency and the subsequent development of SMON by the co-administration 10 of vitamin B<sub>12</sub> and clioquinol. Compositions, formulations, and kits for such co-administration are provided.

The invention also provides methods of treatment and/or prevention of disorders therapeutically or 15 prophylactically responsive to clioquinol administration. Such disorders include but are not limited to infections, e.g. fungal infections, amoebiasis, bacterial infections (*Helicobacter pylori* infections, etc.), neurotoxic injury, infections resulting in peptic ulcer 20 or infectious diarrhea, Alzheimer's disease, and Parkinson's disease.

By way of example, but not limitation, compositions and methods of the invention can be indicated for: 1) patients diagnosed with Alzheimer's disease or 25 Parkinson's disease at any clinical stage of the disease, 2) the prevention of Alzheimer's disease or Parkinson's disease in patients with early or prodromal symptoms or signs, and 3) the delay of the onset or evolution or aggravation of the symptoms and signs of 30 Alzheimer's disease or Parkinson's disease. The methods and compositions of the invention will be, for example, useful for the treatment of Alzheimer's disease or Parkinson's disease, the improvement or alleviation of any symptoms and signs of Alzheimer's disease or 35 Parkinson's disease, the improvement of any pathologi-

cal or laboratory findings of Alzheimer's disease or Parkinson's disease, the delay of the evolution of Alzheimer's disease or Parkinson's disease, the delay of onset of any Alzheimer's disease or Parkinson's disease, symptoms and signs, the prevention of occurrence of Alzheimer's disease or Parkinson's disease, and the prevention of the onset of any of the symptoms and signs of Alzheimer's disease or Parkinson's disease.

The subject, or patient, is an animal, e.g. a mammal, and is preferably human, and can be a fetus, child, or adult.

#### Treatment of Alzheimer's Disease

In a specific embodiment, the invention provides a method of treating or preventing Alzheimer's disease with clioquinol in combination with vitamin B<sub>12</sub>, administered concurrently or sequentially, thereby achieving therapeutic efficacy while inhibiting detrimental side effects of clioquinol administration. Clioquinol is believed to have the ability to penetrate the blood-brain barrier, to effectively chelate heavy metals to prevent the aggregation of amyloid, and to redissolve amyloid deposit. It could not have been predicted that clioquinol had the ability to redissolve zinc precipitated A $\beta$ .

The following proposed mechanism of action of the invention is not intended to limit the invention to said mechanism. At present, applicant believes that clioquinol and A $\beta$  competitively chelate zinc and other heavy metals. Clioquinol is regarded as the stronger chelator and will, therefore, predominately capture the heavy metal ions. Thus, zinc bound to A $\beta$  in A $\beta$  aggregates will be bound by clioquinol. The removal of zinc by clioquinol will resolubilize the A $\beta$  protein. The

complex of clioquinol and zinc should penetrate the blood-brain-barrier and be cleared from the organism.

The dose of clioquinol optimal *in vivo* for the resolubilisation of A $\beta$  can be determined by a physician upon conducting routine experiments. An example of such an experiment is the monitoring of soluble A $\beta$  in the cerebrospinal fluid (CSF) (WO 93/10459, dated May 27, 1993 of University of Melbourne). Beginning with relatively low doses (10-25 mg/day), a physician can monitor the amount of solubilized A $\beta$  in the patient's CSF. If there is no increase in solubilized A $\beta$  in response to the clioquinol administration, indicative of resolubilization of zinc-A $\beta$  aggregates, the dosage can be raised until such an increase is observed.

Prior to administration to humans, the efficacy is preferably shown in animal models. Any animal model for Alzheimer's disease known in the art can be used.

#### Treatment of Parkinson's Disease

In a specific embodiment, the invention provides a method of treating or preventing Parkinson's disease with clioquinol in combination with vitamin B<sub>12</sub>, administered concurrently or sequentially, thereby achieving therapeutic efficacy while inhibiting detrimental side effects of clioquinol administration.

The invention provides a use of clioquinol and vitamin B<sub>12</sub> for the manufacture of a pharmaceutical composition for the treatment of Parkinson's disease. This composition is believed to have the ability to penetrate the blood-brain barrier, to effectively chelate heavy metals to prevent the generation of hydroxyl radicals and hydrogen peroxide, and to prevent oxidation of molecules found in the brain, including MPTP.

A role for an increase of oxidative stress in the mechanism of Parkinson's disease development is implicated by the finding that in all stages of Parkinson's disease, patients exhibit increased iron levels, 5 decreased ferritin levels, and reduced glutathione (glutathione acts as a free radical scavenger by serving as a reducing agent).

Without intending to be bound by any mechanism, the aim of using a chelating agent such as clioquinol 10 is to chelate or clear the iron that has been accumulated in the brain and, in particular, in the substantia nigra. In this way, iron catalyzed free radical production can be minimized and the oxidation of MPTP or other molecules inhibited.

15 Prior to administration to humans, the efficacy is preferably shown in animal models. Any animal model for Parkinson's disease known in the art can be used.

#### Pharmaceutical Compositions

20 The pharmaceutical compositions according to the present invention preferably comprise one or more pharmaceutical acceptable carriers and the active constituents. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredi- 25 ents of the composition and not deleterious to the recipients thereof. In a preferred embodiment, the clioquinol and/or vitamin B<sub>12</sub> in the pharmaceutical composition is purified.

It will be appreciated that the amounts of clio- 30 quinol and vitamin B<sub>12</sub> required for said treatment or prevention will vary according to the route of administration, the disorder to be treated, the condition, age, the file history of the subject, and the galenic formulation of the pharmaceutical composition, etc.



Preferably, the clioquinol used in the invention is of high purity, such as that provided by Spectrum Quality Products, specification: USP 23; however any suitable preparation of clioquinol can be used in the 5 methods and composition of the invention.

Preferably, the vitamin B<sub>12</sub> used in the invention is of high purity, such as that provided by Hoffman La Roche, Ltd., specification: pharmaceutical grade; however any suitable preparation of vitamin B<sub>12</sub> can be 10 used in the methods and compositions of the invention.

In general, a suitable therapeutically effective amount of clioquinol in the pharmaceutical composition is, for example, 5 to 250 mg, preferably 10 to 50 mg. A suitable amount of vitamin B<sub>12</sub>, effective to inhibit 15 clioquinol related side effects, in the pharmaceutical composition is, for example, 5 µg to 2 mg, preferably 0.5 to 1 mg. The actually administered amounts of clioquinol and vitamin B<sub>12</sub> may be decided by a supervising physician. Clioquinol and vitamin B<sub>12</sub> can be in 20 the same composition for administering in combination concurrently, or in different compositions for administering concurrently but separately, or sequentially.

Therapeutic formulations include those suitable for parenteral (including intramuscular and intra- 25 venous), oral, rectal or intradermal administration, although oral administration is the preferred route. Thus, the pharmaceutical composition may be formulated as tablets, pills, syrups, capsules, suppositories, formulations for transdermal application, powders, 30 especially lyophilized powders for reconstitution with a carrier for intravenous administration, etc.

The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. The carriers in the pharmaceutical 35 composition may comprise a binder, such as

microcrystalline cellulose, polyvinylpyrrolidone (polyvidone or povidone), gum tragacanth, gelatine, starch, lactose or lactose monohydrate; a disintegrating agent, such as alginic acid, maize starch and the like; a lubricant or surfactant, such as magnesium stearate, or sodium lauryl sulphate; a glidant, such as colloidal silicon dioxide; a sweetening agent, such as sucrose or saccharin; and/or a flavouring agent, such as peppermint, methyl salicylate, or orange flavouring.

Therapeutic formulations suitable for oral administration, e.g. tablets and pills, may be obtained by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by mixing clioquinol and vitamin B<sub>12</sub>, and compressing this mixture in a suitable apparatus into tablets having a suitable size. Prior to the mixing, the clioquinol may be mixed with a binder, a lubricant, an inert diluent and/or a disintegrating agent and the vitamin B<sub>12</sub> may be mixed with a diluent, a lubricant and/or a surfactant.

In a preferred embodiment, free-flowing clioquinol powder is mixed with a binder, such as microcrystalline cellulose, and a surfactant, such as sodium lauryl sulphate, until a homogeneous mixture is obtained. Subsequently, another binder, such as polyvidone, is transferred to the mixture under stirring. When a uniform distribution is obtained an aqueous solution of vitamin B<sub>12</sub> is added under constant stirring. This mixture is passed through granulating sieves and dried by desiccation before compression into tablets in a standard compressing apparatus.

In a second preferred embodiment, free-flowing clioquinol powder is mixed with surfactants and/or emulsifying agents, such as Sapamine® (N-(4'-stearoyl amino phenyl)-trimethylammonium methyl sulphuric acid)

and lactose monohydrate until a uniform distribution of the constituents is obtained. A second preparation containing a disintegrating agent, such as maize starch, is added to the clioquinol mixture under continuous stirring. Such a second preparation may be prepared by adding excess boiling water to a maize starch suspended in cold water. An aqueous solution of vitamin B<sub>12</sub> is added to the clioquinol mixture. The final mixture is granulated and dried as above and mixed with maize starch and magnesium stearate and finally compressed into tablets in a standard apparatus.

A tablet may be coated or uncoated. An uncoated tablet may be scored. A coated tablet may be coated with sugar, shellac, film or other enteric coating agents.

Therapeutical formulations suitable for parenteral administration include sterile solutions or suspensions of the active constituents. An aqueous or oily carrier may be used. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Formulations for parenteral administration also include a lyophilized powder comprising clioquinol and vitamin B<sub>12</sub> that is to be reconstituted by dissolving in a pharmaceutically acceptable carrier that dissolves vitamin B<sub>12</sub> and clioquinol, e.g. an aqueous solution of carboxymethyl-cellulose and lauryl sulphate.

When the pharmaceutical composition is a capsule, it may contain a liquid carrier, such as a fatty oil, e.g. cacao butter.

Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour,

chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides.

In yet another embodiment, the therapeutic compound can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, *supra*; Sefton, CRC Crit. Ref. Biomed. Eng. 14: 201 (1987); Buchwald et al., Surgery 88: 507 (1980); Saudek et al., N. Engl. J. Med. 321: 574 (1989)). In another embodiment, polymeric materials can be used (see Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J. Macromol. Sci. Rev. Macromol. Chem. 23: 61 (1983); see also Levy et al., Science 228: 190 (1985); During et al., Ann. neurol. 25: 351 (1989); Howard et al., J. Neurosurg. 71: 105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, i.e., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in Medical Applications of Controlled Release, *supra*, vol. 2, pp. 115-138 (1984)).

Other controlled release systems are discussed in the review by Langer (Science 249: 1527-1533 (1990)).

In one embodiment of the pharmaceutical composition according to the invention, the active constituents, i.e. vitamin B<sub>12</sub> and clioquinol, are comprised as separate entities. The two entities comprising vitamin

B<sub>12</sub> and clioquinol, respectively, may be administered simultaneously or sequentially. For example, clioquinol can be administered, followed by vitamin B<sub>12</sub> administered within a day, week, or month of clioquinol administration. If the two entities are administered sequentially, the entity comprising clioquinol is preferably administered for one to three weeks followed by a wash out period of one to four weeks during which the entity comprising vitamin B<sub>12</sub> is administered, but not the entity comprising clioquinol. After the wash out period, the treatment can be repeated.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form described by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

Other features and advantages of the invention will be apparent from the following examples, which in conjunction with the accompanying drawings illustrate by way of example the principles of the invention.

## EXAMPLES

### Example 1

The influence of clioquinol on vitamin B<sub>12</sub> *in vivo* was studied.

Six week old male mice were divided into two groups, a control group and a group which was injected with clioquinol (50 mg/kg/day). After three days, all of the mice were injected with [<sup>57</sup>Co]-cyanocobalamine. After two days, the animals were sacrificed, and the

brain, liver and kidney were dissected and counted in a gamma-counter as thousand cpm/g tissue (wet weight)  $\pm$  SEM. The radioactivities in each of the groups are stated in Table 1 below:

5

Table 1

Treatment	Brain	Liver	Kidney
Control	9.4 $\pm$ 0.9	97 $\pm$ 8	895 $\pm$ 207
Clioquinol	8.4 $\pm$ 1.5	85 $\pm$ 21	252 $\pm$ 61

10

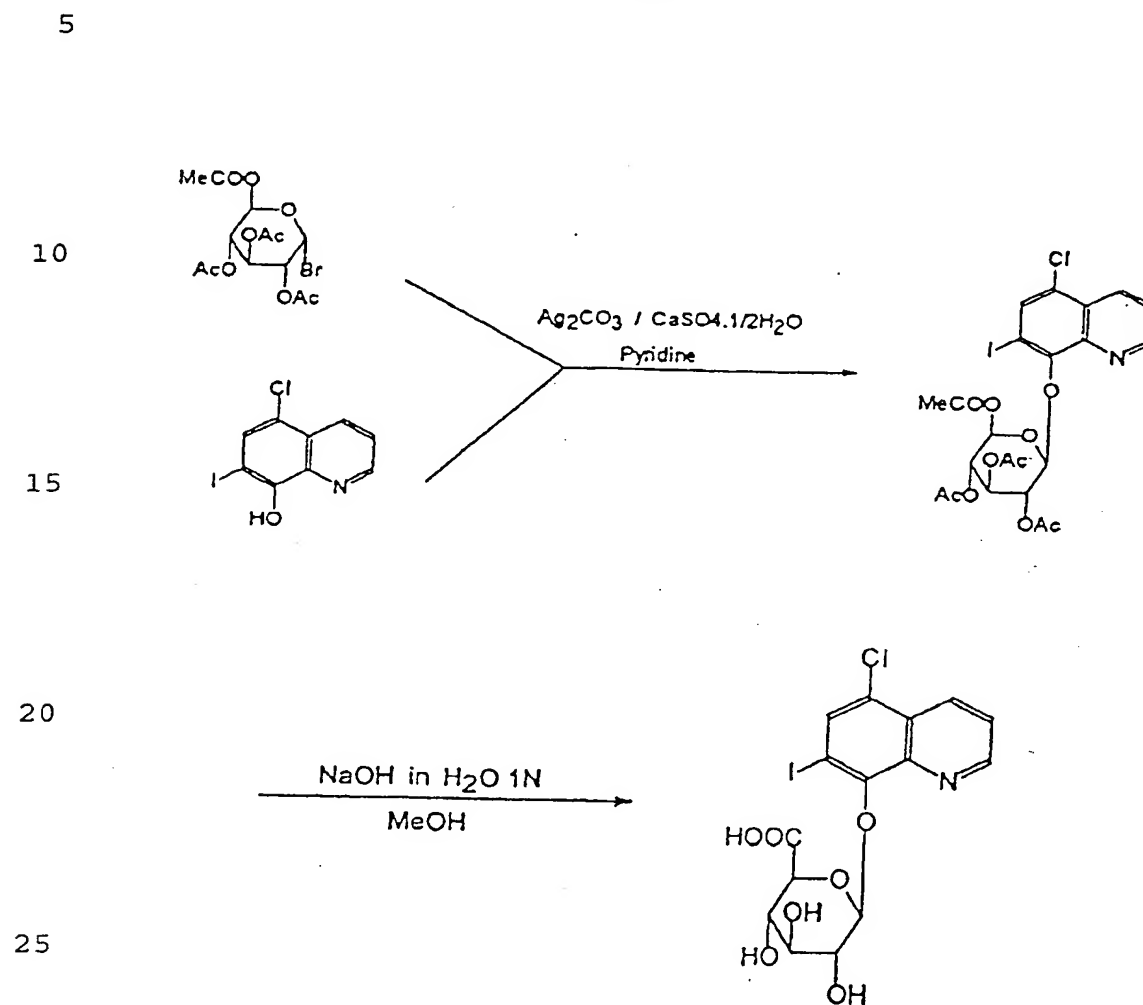
A comparison of the results show that there were no significant changes in the amount of radioactivity accumulation in the brain and the liver. A reduction in the amount of vitamin B<sub>12</sub> trapped in the kidneys was  
15 apparent. This result suggest that clioquinol interferes with the vitamin B<sub>12</sub> metabolism in the kidneys but not elsewhere in the body.

#### Example 2

20 In this example, a metabolite of clioquinol was synthesized.

It is known that the clioquinol is excreted through the kidneys as glucuronide derivatives of clioquinol (Kotaki H., et al.: "Enterohepatic circulation of clioquinol in the rat", J. Pharmacobiodyn. 1984  
25 June; 7(6): 420-5 and Jurima M. et al.: "Metabolism of 14C-iodochlorhydroxyquinoline in the dog and the rat", J. Pharmacobiodyn. 1984 March; 7(3): 164-70). The transformation of clioquinol to the corresponding glucuronide  
30 curonide presumable takes place in the liver. Following the formation of clioquinol glucuronide in the liver it is eventually transferred to the kidneys for excretion in the urine.

A glucuronide derivative of clioquinol in the form of methyl (5-chloro-7-iodo-quinolyl-2',3',4'-tri-O-acetyl-glycopyranosid)uronate was prepared according to the following reaction scheme:



A mixture of 5-chloro-8-hydroxy-7-iodo-quinoline (50 mg, 0.164 mmol), methyl 1-bromo-1-deoxy-2,3,4-tri-O-acetyl-D-glucopyranosiduronate (65 mg, 0.164 mmol), 35  $\text{CaSO}_4 \cdot \text{H}_2\text{O}$  (35 mg) and pyridine (1.5 ml) was stirred at

room temperature for 20 min. Freshly prepared  $\text{Ag}_2\text{CO}_3$  (35 mg) was added to the reaction mixture and the suspended solution was stirred at room temperature for 20 hours in the dark. Subsequently, the reaction product was deacetylated by 1 N aqueous NaOH.

The reaction mixture was diluted with  $\text{CH}_2\text{Cl}_2$  (10 ml), filtered and the solvent evaporated under reduced pressure. The above product was purified by flash chromatography (TLC:  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  99/1, eluent:  $\text{CH}_2\text{Cl}_2/-$  MeOH 99.5/0.5).

NMR (400MHz,  $\text{CDCl}_3$ ) 2.04 (s, 3H, Ac), 2.09 (s, 3H, Ac), 2.13 (s, 3H, Ac), 3.68 (s, 3H, Me), 3.99 (d, 1H, 5'-H), 5.40-5.52 (m, 3H, 2',-3',-4'-H), 6.29 (d, 1H, 1'-H), 7.56 (m, 1H, 3H), 7.99 (s, 1H, 6-H), 8.52 (d, 1H, 4-H), 8.93 (s, 1H, 2-H).

This compound is referred to as clioquinol glucuronide in the following.

### Example 3

The interaction of vitamin  $\text{B}_{12}$  with clioquinol glucuronide as prepared in example 2, was studied using nuclear magnetic resonance (NMR) spectroscopy.

As the clioquinol glucuronide is soluble in water, the study was undertaken in buffered water at pH=6.5. Three different solutions were prepared and their  $^1\text{H}$  NMR spectra were recorded in a DRX 400 MHz spectrophotometer at 20°C. Solution a) contained free vitamin  $\text{B}_{12}$  (hydroxycobalamin) in a concentration of 2.6 mM. Solution b) contained a mixture of 2.6 mM vitamin  $\text{B}_{12}$  and 10mM clioquinol glucuronide (mole ratio of about 1:4). Solution c) contained 10 mM clioquinol glucuronide.

In Fig. 1 the spectra of the three solutions are presented for the aromatic region (5.5-9.8 ppm). The differences are quite small but obvious in the expan-



sion shown of Fig. 2 and Fig. 3, respectively. Some of the resonances of vitamin B<sub>12</sub> (corresponding to the benzimidazole moiety) have shifted (see Fig. 2), and the same is observed for two resonances of the clio-  
5 quinol glucuronide (corresponding to the quinoline moiety) (see Fig. 3).

The results suggest an interaction between clio-quinol glucuronide and vitamin B<sub>12</sub>, possibly of a hydrophobic nature between the benzimidazole moiety of  
10 the vitamin B<sub>12</sub> and the quinoline moiety of clioquinol glucuronide.

The hydrophobic binding of vitamin B<sub>12</sub> to clio-quinol glucuronide is believed to cause the vitamin B<sub>12</sub> to be excreted from the body together with clioquinol  
15 glucuronide, thus preventing resorption of vitamin B<sub>12</sub>, which would eventually lead to a vitamin B<sub>12</sub> deficiency. Therefore, vitamin B<sub>12</sub> deficiency is believed to be, at least to some extent, the underlying cause of SMON.

20

#### Example 4

Preparation of a pharmaceutical composition comprising clioquinol and vitamin B<sub>12</sub>.

250 g of clioquinol (5-chloro-7-iodo-8-quinoline)  
25 was mixed for a period 3 minutes with 1657.5 g micro-crystalline cellulose and 20 g sodium lauryl sulphate. An aqueous solution of 25 g polyvidone and an aqueous solution of 5.0 g vitamin B<sub>12</sub> (cyanocobalamine) was added to the clioquinol containing powder mixture under  
30 stirring. This mixture was passed through granulating sieves (2,5 mm) and desiccated for a period of 20 hours at 40°C. The dry mixture (sieved at 1.25 mm) was blended with 30 g sodium lauryl sulphate and 12,5 g magnesium stearate. This mixture was formed into

tablets having a diameter of 8.0 mm and a weight of 200 mg.

#### Example 5

5 Preparation of a pharmaceutical composition comprising clioquinol and vitamin B<sub>12</sub>.

250 g of clioquinol (5-chloro-7-iodo-8-quinoline) was mixed with 200 g sapamine<sup>®</sup> (N-(4'-stearoyl amino-phenyl)-trimethylammonium methyl sulphuric acid) and  
10 1025 g lactose mono-hydrate for a period of 5 minutes. 300 g of boiling water was added in one go to a mixture of 100 g maize starch in 100 g cold water. The maize suspension, cooled to 40°C was added to the clioquinol containing powder mixture under continuous stirring.  
15 Subsequently, an aqueous solution of 5 g vitamin B<sub>12</sub> was added. The mixture was granulated using a 2.5 mm sieve and desiccated for 18 hours at 40°C. The dry granules were mixed with 400 g maize starch and 20 g magnesium stearate. The final mixture was formulated  
20 into tablets having a diameter of 8.0 mm and a weight of 200 mg.

#### Example 6

The resolubilization of amyloid aggregates by  
25 clioquinol was studied.

5.3 g of clioquinol was suspended with agitation in 200 ml of n-decane. The undissolved material was allowed to settle. Weighing the dried undissolved clioquinol after blowing off the decane indicated that  
30 only 2% of the clioquinol dissolved in the decane. 100 ml of the (light yellow) supernatant was agitated together with 100 ml of PBS pH 7.4 and the phases allowed to separate. The PBS (lower phase) was collected and filtered to remove the residue which formed  
35 at the phase interface upon extraction with the organic

solvent. Assuming that 2% of the clioquinol dissolved in the n-decane, and assuming that the partitioning coefficient is 1/1750 with PBS at 1:1 mixtures of decane/cliioquinol, the concentration of clioquinol in the PBS is 800 nM.

Brain specimens were obtained for which a histopathological diagnosis of Alzheimer's disease was confirmed. Duplicate 0.5 g specimens of frontal lobe neocortex were homogenized in 3 ml of the clioquinol/PBS solution at 100%, 10% and 1% of the final PBS/cliioquinol extract and PBS alone.

The homogenates were centrifuged at 150,000 xg for thirty minutes and the supernatants collected and held on ice (fraction "S"). The pellets were subjected to an identical homogenization and centrifugation regime and the resulting supernatants again collected (fraction "P").

1 ml of each supernatant was treated with 200  $\mu$ l of ice cold 10% TCA to precipitate total protein including A $\beta$ . The resulting pellet was washed once with 100% ethanol and resuspended in 100  $\mu$ l of TBS (tris 20 mM, NaCl 150 mM, pH 7.4).

7.5  $\mu$ l of sample (S or P) was boiled for 5 min with an equal volume of tris-tricine sample buffer containing 4% SDS and loaded onto a Novex pre-cast 10-20% tris-tricine gel followed by Western transfer onto nitrocellulose. Signal for A $\beta$  was detected using mAb WO2 (raised against residues 5-16 of A $\beta$ ) and visualized using ECL. The sensitivity of the detection system is 5-10 pg.

To validate the TCA precipitation for A $\beta$  1  $\mu$ g of synthetic A $\beta$  1-40 was added to 1 ml of PBS containing 10% BSA and the solution was treated as above. Signal for A $\beta$  was detected in the precipitated pellet but not in the supernatant.

The result is indicated in Fig. 4.

As can be deduced from Fig. 4, the clioquinol was effective in promoting the solubilization of A $\beta$  in the concentrations tested. Furthermore, the optimal concentration was found to be "10%", indicating that one of the aggregation forms of A $\beta$ , presumably the dimer, is more soluble in PBS than others.

While data are shown only for one specimen in Fig. 1, data for 19 other specimens all indicate the same tendency, viz. that clioquinol is effective in promoting the solubilization of A $\beta$ .

#### Example 7

A comparison of the chelating abilities of EDTA and clioquinol was made.

Samples of 10 mg synthetic A $\beta$  were placed in microtitre wells and caused to aggregate by the addition of 25  $\mu$ M ZnCl<sub>2</sub>. The aggregates were then transferred to a 0.2  $\mu$ m nylon membrane by filtration. The aggregates were washed with 200  $\mu$ l TBS alone, TBS containing 2  $\mu$ M EDTA, and TBS containing 2  $\mu$ M clioquinol. The membrane was fixed, probed with the anti A $\beta$  monoclonal antibody 6E10 and developed for exposure to ECL-film. The transmittance of the ECL-film was measured and the relative signal strength calculated based on 100% for TBS alone. The relative signal strength was 66% for EDTA and 49% for clioquinol.

The results indicate that clioquinol is a better chelator for zinc precipitated A $\beta$  than EDTA.

#### Example 8

The ability of clioquinol to resolubilize zinc-aggregated A $\beta$  was assessed.

A 2.5  $\mu$ M solution of A $\beta$  in TBS at a pH of 7.4 was prepared. 95% of the A $\beta$  was maintained in a soluble

state. Addition of 30  $\mu$ M zinc resulted in precipitation of the soluble A $\beta$  and only 43% was maintained in solution. The subsequent addition of 120  $\mu$ M clioquinol to the zinc precipitated A $\beta$  resulted in an increase of 5 soluble A $\beta$  to 70%.

The results indicate that clioquinol is able to redissolve zinc precipitated A $\beta$ .

#### Example 9

10 A patient suffering from Alzheimer's disease is treated with the tablet described in Example 5 as follows:

In the first period of treatment, one tablet a day for two weeks is administered, followed by a two week  
15 wash-out period, during which vitamin B<sub>12</sub> administration alone is continued. The tablet containing vitamin B<sub>12</sub> alone is formulated identically to the tablet described in Example 5, except that clioquinol is omitted. After the wash-out period, the first period is  
20 repeated. During the treatment, the amount of solubilized A $\beta$  in the patient's cerebrospinal fluid (CSF) is monitored. If there is no increase in solubilized A $\beta$  in response to the clioquinol administration, indicative of resolubilization of zinc-A $\beta$   
25 aggregates, the dosage is raised to two tablets once a day. An observed increase of solubilized A $\beta$  in the patient's CSF indicates that the treatment is effective at resolubilizing A $\beta$ . Preferably, two more cycles of clioquinol in combination with vitamin B<sub>12</sub> followed by  
30 vitamin B<sub>12</sub> alone are administered.

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention, and all such  
5 modifications, as would be obvious to a person skilled in the art, are intended to be included in the scope of the following claims.

## P A T E N T   C L A I M S

1. Pharmaceutical composition comprising clioquinol, vitamin B<sub>12</sub>, and, optionally, pharmaceutically acceptable carriers.
- 5        2. Pharmaceutical composition according to claim 1, wherein the amount of clioquinol is 5 to 250 mg.
3. Pharmaceutical composition according to claim 1 or 2, wherein the amount of clioquinol is 10 to 50 mg.
- 10       4. Pharmaceutical composition according to any of the claims 1 to 3, wherein the amount of vitamin B<sub>12</sub> is 5 µg to 2 mg.
5. Pharmaceutical composition according to any of the claims 1 to 4, wherein the amount of vitamin B<sub>12</sub> is  
15 0.5 mg to 1 mg.
6. Pharmaceutical composition according to any of the claims 1 to 5, wherein clioquinol and vitamin B<sub>12</sub> are comprised as separate entities.
7. Pharmaceutical composition according to any of  
20 the claims 1 to 6, wherein the entity comprising vitamin B<sub>12</sub> is administered substantially simultaneously with the administration of the entity comprising clioquinol.
8. Pharmaceutical composition according to any of  
25 the claims 1-7, wherein the two entities are administered sequentially.
9. Pharmaceutical composition according to any of the claims 1 to 8, wherein the entity comprising clioquinol is administered for one to three weeks followed  
30 by a period of one to four weeks during which the entity comprising vitamin B<sub>12</sub> is administered and clioquinol is not administered.
10. Pharmaceutical composition according to any of the claims 1 to 9, wherein the pharmaceutical composi-

tion is formulated for parenteral, oral or intradermal administration.

11. Use of vitamin B<sub>12</sub> and clioquinol for the manufacture of a pharmaceutical composition for the treatment or prevention of a disorder responding to clioquinol administration.

12. Use according to claim 11, wherein the amount of clioquinol is 5 to 250 mg.

13. Use according to claim 12, wherein the amount of clioquinol is 10 to 50 mg.

14. Use according to any of the claims 11 to 13, wherein the amount of vitamin B<sub>12</sub> is 5 µg to 2 mg.

15. Use according to claim 14, wherein the amount of vitamin B<sub>12</sub> is 0.5 mg to 1 mg.

16. Use according to any of the claims 11 to 14, wherein the disorder is an infection caused by bacteria, protozoa, fungi or virus.

17. Use according to claim 16, wherein the infection is amoebiasis or infectious diarrhea.

18. Use according to claim 16, wherein the infection is caused by *Helicobacter pylori*.

19. Use according to any of the claims 11 to 18, wherein the disorder is Alzheimer's disease.

20. Use according to any of the claims 11 to 19, wherein the disorder is Parkinson's disease.

21. Use according to any of the claims 11 to 20, wherein the pharmaceutical composition comprises vitamin B<sub>12</sub> and clioquinol as separate entities.

22. Use according to claim 21, wherein the entity comprising vitamin B<sub>12</sub> is administered substantially simultaneously with the administration of the entity comprising clioquinol.

23. Use according to claim 21, wherein the two entities are administered sequentially.



24. Use according to claim 23, wherein the entity comprising clioquinol is administered for one to three weeks, followed by a period of one to four weeks during which the entity comprising vitamin B<sub>12</sub> is administered 5 and clioquinol is not administered.

25. Use according to any of the claims 11 to 24, wherein the pharmaceutical composition is formulated for parenteral, oral or intradermal administration.

26. A kit comprising in one or more containers a 10 therapeutically effective amount of clioquinol and a therapeutically effective amount of vitamin B<sub>12</sub>.

27. Pharmaceutical composition according to any of the claims 1 to 6, wherein the pharmaceutical composition is formulated as a pill.

15 28. Pharmaceutical composition according to any of the claims 1 to 6, wherein the pharmaceutical composition is formulated as a tablet.

29. Pharmaceutical composition according to any of the claims 1 to 6, wherein the pharmaceutical composition 20 is formulated as a capsule.

30. Pharmaceutical composition according to any of the claims 1 to 6, wherein the pharmaceutical composition is formulated as a suppository.

31. Pharmaceutical composition according to any of 25 the claims 1 to 6, wherein the pharmaceutical composition is formulated for oral administration with an emulsifying agent.

32. The pharmaceutical composition according to claim 28, wherein the emulsifying agent is N-(4'- 30 stearoyl amino phenyl) trimethylammonium methyl sulphuric acid.

33. A method of treating a subject with a disease or disorder responsive to clioquinol administering comprising administering to the subject clioquinol and 35 vitamin B<sub>12</sub>.

34. A method of treating a subject having or suspected of having a disease or disorder responsive to clioquinol administration while inhibiting detrimental side effects comprising administering to the subject  
5 (a) a therapeutically effective amount of clioquinol, and (b) an amount of vitamin B<sub>12</sub> effective to inhibit a detrimental side effect of clioquinol administration.

35. The method according to claims 34 wherein the clioquinol and vitamin B<sub>12</sub> are comprised in a single  
10 pharmaceutical composition.

36. The method according to claim 34 wherein the clioquinol and vitamin B<sub>12</sub> are administered sequentially.

37. The method according to claim 34 wherein the  
15 clioquinol and vitamin B<sub>12</sub> are administered substantially simultaneously.

38. The method according to claim 36 wherein a pharmaceutical composition comprising clioquinol is administered for one to three weeks, followed by a  
20 period of one to four weeks during which a pharmaceutical composition comprising vitamin B<sub>12</sub> is administered and clioquinol is not administered.

39. The method according to claim 34 wherein the subject is human.

25 40. The method according to claim 39 wherein the disease or disorder is Alzheimer's disease.

41. The method according to claim 39 wherein the disease or disorder is Parkinson's disease.

42. The method according to claim 39 wherein the  
30 disease or disorder is a bacterial, protozoal, viral or fungal infection.

43. The method according to claim 39 wherein the disease or disorder is an infection by *Helicobacter pylori*.

44. The method according to claim 39 wherein the  
35 disease or disorder is caused by rota-virus.

45. The method according to claim 39 wherein the disease or disorder is amoebiasis.

46. The method according to claim 39 wherein the disease or disorder is infectious diarrhea.

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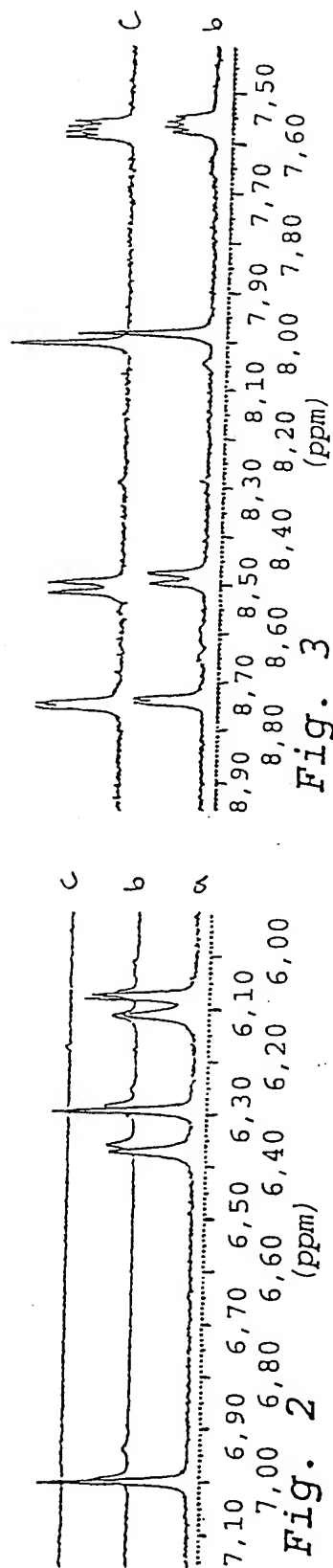
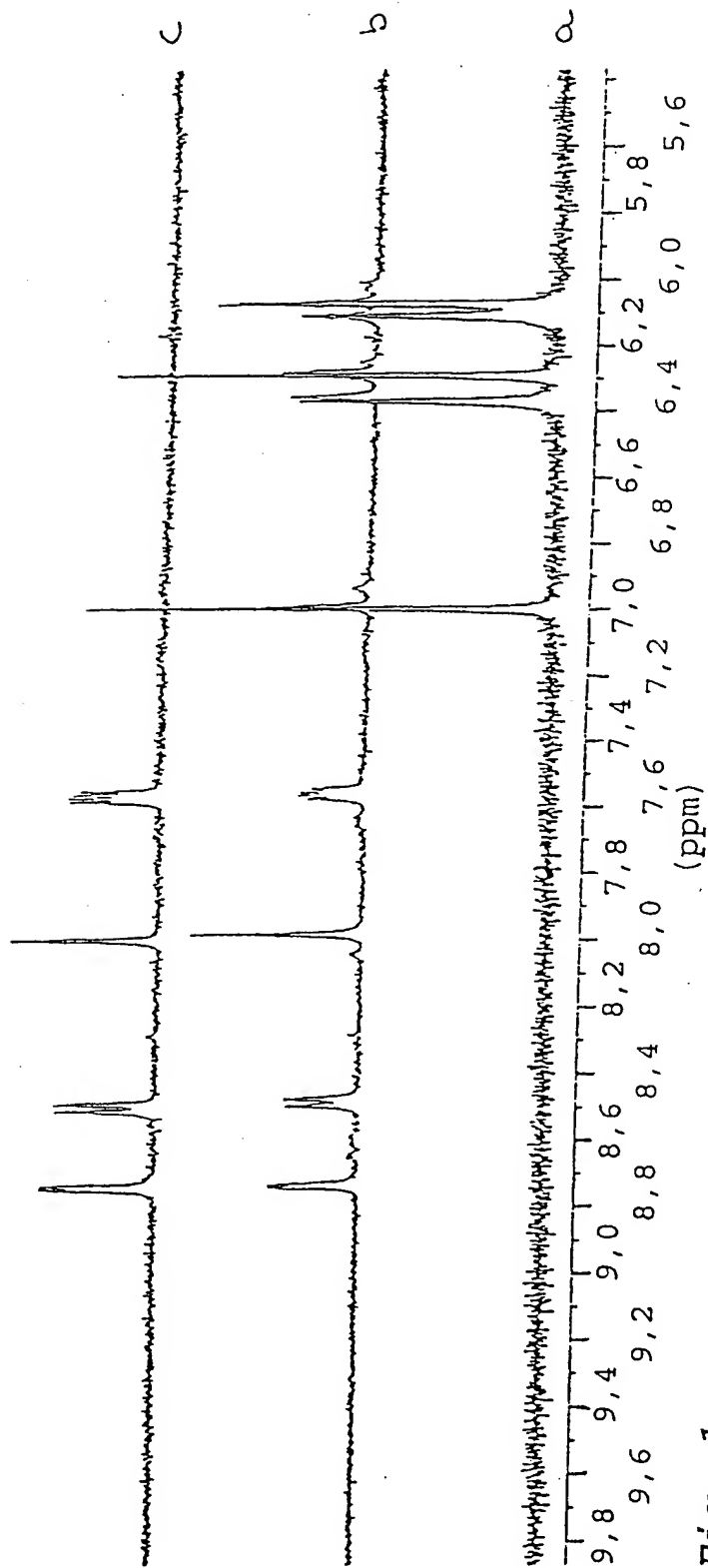


Fig. 3

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Solubilisation of  $\beta$ Amyloid with  
Clioquinol

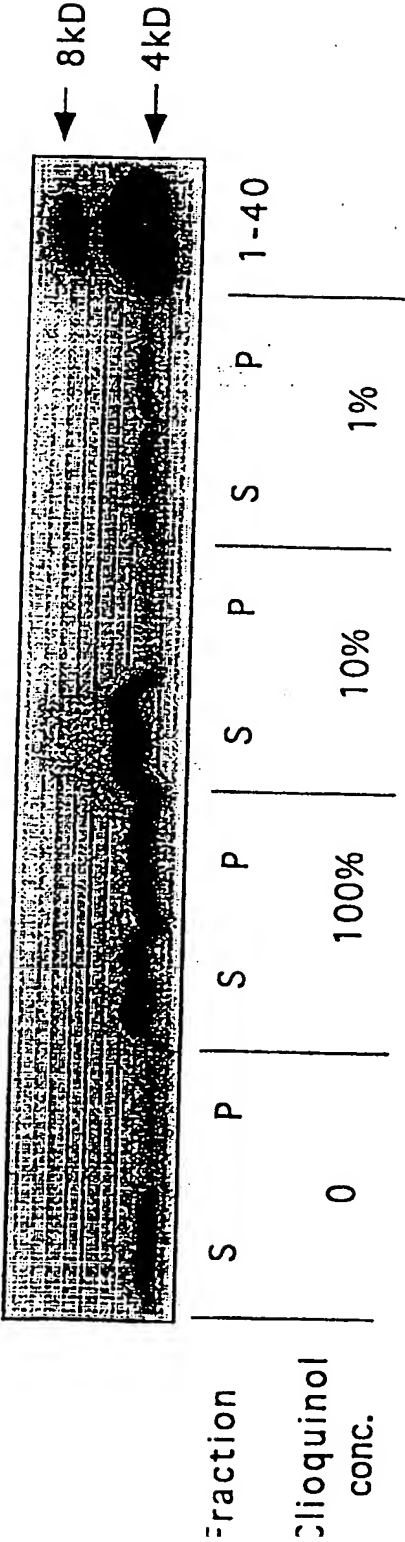


FIG. 4

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# INTERNATIONAL SEARCH REPORT

International Application No

PCT/IB 98/02115

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K31/68 //(A61K31/68,31:47)

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 99 09981 A (XILINAS MICHEL ; GEROLYMATOS PANAYOTIS NIKOLAS (GR); GEROLYMATOS P) 4 March 1999 see abstract see page 9, line 32 - page 11, line 31 see page 13, line 22 - page 14, line 13 see page 17, line 21 - page 18, line 3; claims 12,13,26-37,49-64; examples 5,7 ---	1-46
P, X	WO 98 06403 A (GEROLYMATOS PANAYOTIS NIKOLAS ; GEROLYMATOS P N SA (GR)) 19 February 1998 cited in the application see abstract see page 4, line 28 - page 5, line 9 see page 6, line 1 - page 7, line 16; claims 1-14; examples 4,5 --- -/--	1-46

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
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Date of the actual completion of the international search

16 June 1999

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# INTERNATIONAL SEARCH REPORT

International Application No

PCT/IB 98/02115

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>GB 951 992 A (CIBA) 11 March 1964</p> <p>see page 1, left-hand column, line 1 - right-hand column, line 30 see page 2, right-hand column, line 55 - page 3, right-hand column, line 24; claims; example 5</p>	<p>1,6-11, 16,17, 21,25, 26,31, 33-35,37</p>
X	<p>US 3 178 343 A (CIBA) 13 April 1965</p> <p>see column 1, line 1 - line 72; example 1 see column 3, line 55 - column 4, line 33; claims</p>	<p>1,6-11, 16,17, 21,25, 26,31, 33-35,37</p>
A	<p>WO 95 31199 A (GEROLYMATOS P N SA ;LEGAKIS NICOLAOS (GR)) 23 November 1995 cited in the application see the whole document</p>	<p>1-46</p>
A	<p>REYNOLDS J E F: "MARTINDALE, THE EXTRA PHARMACOPOEIA, PASSAGE TEXT" MARTINDALE - THE EXTRA PHARMACOPOEIA, no. ED. 30, page 511 XP002047208 REYNOLDS J E F see the whole document</p>	<p>1-46</p>
A	<p>L.M. MCEWEN: "Neuropathy after clioquinol" BRITISH MEDICAL JOURNAL, vol. 4, no. 780, 1971, pages 169-170, XP002106152 see the whole document</p>	<p>1-46</p>
A	<p>H. TAGUCHI ET AL.: "Vitamin B12 levels of cerebrospinal fluid in patients with a variety of neurological disorders" J. NUTR. SCI. VITAMINOL., vol. 23, no. 4, 1977, pages 299-304, XP002106153 see the whole document</p>	<p>1-46</p>

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB 98/02115

### Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 33-46  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claims 33-46  
are directed to a method of treatment of the human/animal  
body, the search has been carried out and based on the alleged  
effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such  
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

### Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all  
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment  
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report  
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is  
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/IB 98/02115

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